EGF-CFC GENES AND AXIAL MIDLINE FORMATION IN THE MOUSE.

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The *EGF-CFC* gene family encodes small extracellular proteins that are likely to function as co-receptors for Nodal, a secreted morphogen of the transforming growth factor-beta (TGF) family that is required for several fundamental aspects of establishment of the vertebrate body plan. To date, two members of the family (*Cripto* and *Cryptic*) have been isolated in mammals, while only a single *EGF-CFC* gene has been found in other vertebrates, corresponding to chick *Cripto,* frog *FRL-1,* and zebrafish *one-eyed pinhead (oep)*. We have previously shown through targeted gene disruption in the mouse that the *EGF-CFC* gene *Cripto* is required for embryonic mesoderm and definitive endoderm formation, as well as correct orientation of the anterior-posterior axis. In contrast, we have found that the related gene *Cryptic* is essential for left-right (L-R) axis patterning, and is specifically required for asymmetric expression of L-R pathway genes in the lateral plate mesoderm. These findings have supported a role for EGF-CFC proteins as essential components of the Nodal signaling pathway, and have provided insights into the functions of Nodal in embryonic patterning.

To investigate later functions of *Cripto* in embryogenesis, we have circumvented the early embryonic lethality of *Cripto* null mutations by pursuing a tissue-specific targeting approach using *Cre/loxP*-mediated recombination. In the process of creating a floxed allele of *Cripto* for this purpose, we have generated a partial loss-of-function (hypomorphic) allele, $Cripto^{3\text{-}loxP}$, which contains a *PGK-neo* cassette inserted between exons 5 and 6. When we cross these $Cripto^{3\text{-}loxP}$/+ mice with $Cripto^{lacZ}$/+ mice, which are heterozygous for a null allele, we find that the resulting $Cripto^{3loxP}/Cripto^{lacZ}$ progeny display a severe axial midline defect that resembles human holoprosencephaly.

Morphological and histological examination of $Cripto^{3loxP}/Cripto^{lacZ}$ embryos at 8.5 and 9.5 dpc reveals that approximately two-thirds of these embryos display severe cyclopia and reduced forebrain and midbrain structures. These embryos often also show fusions of anterior somites across the midline, indicating the absence of notochord, which is confirmed on histological sections. Furthermore, these embryos frequently display randomized cardiac looping and embryo turning, which we interpret as a L-R laterality defect that is an indirect consequence of defects in the axial midline. In addition, a smaller number of embryos can be recovered at 10.5 and 11.5 dpc with a weaker holoprosencephaly phenotype. Our preliminary marker analysis suggests that forebrain and midbrain markers are still expressed at 9.5 dpc in severely affected $Cripto^{3loxP}/Cripto^{lacZ}$ embryos, but are greatly reduced in their domains of expression. We find that *Otx2,* a marker of forebrain and midbrain, as well as *En1,* which marks midbrain, are expressed in limited regions of all mutant embryos. Consistent with possible defects in prechordal plate and notochord, we observe strongly reduced or patchy expression of *Sonic hedgehog* along the axial midline at 8.5 dpc; unexpectedly, expression of *Shh* in the foregut and hindgut endoderm appears relatively normal. Thus, our analysis reveals that severely affected $Cripto^{3loxP}/Cripto^{lacZ}$ embryos have defects in axial mesendoderm formation. Interestingly, our findings show that reduction of Cripto activity can lead to a “pinhead” phenotype that is reminiscent of the zygotic *oep* mutant phenotype, further supporting the evolutionary conservation of *EGF-CFC* functions.

References:

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